effect on the subsequent development of *T. gambiense* ingested within 24 to 48 hours after the arsenic blood.

III. The feeding of flies on arsenic-containing blood immediately after the imbibition of *T. gambiense* usually prevents further development of the trypanosomes in the fly. In the event of development occurring the strain produced is not arsenic-fast.

IV. Arsenphenylglycin exerts a prophylactic effect in a monkey against infection with *T. gambiense* by positive *G. palpalis*; this effect varies with the dosage employed, and is considerably greater than when the trypanosomes are introduced by direct inoculation of infected blood.

On a Gregarine—Steinina rotundata, *nov. sp.*—*Present in the Mid-Gut of Bird-Fleas of the Genus* Ceratophyllus.

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(Communicated by J. C. Ewart, F.R.S. Received September 28,—Read December 5, 1912.)

[Plate 1.]

The Gregarine described in the following account was first observed by one of us, in autumn, 1909, in the alimentary canal of fleas—*Ceratophyllus styx*, Rothschild—from a sand-martin's nest.* We have since collected, from several localities in the Scottish Lowlands, numerous larval, pupal, and adult specimens of *C. styx*, in which we have observed the various phases of development of the parasite. We have also dissected about 500 fleas of other species, in order to determine whether they also harboured the Gregarine which we had found in *C. styx*. The species examined were *C. farreni*, Rothsch., from nests of the house-martin; *C. gallinæ*, Schrank, from nests of the blue-tit,† the blackbird, the thrush, and the robin;

* The Rev. J. Waterston had previously reported that he had noticed in a specimen of *C. styx*, mounted whole, after having been partially cleared in caustic potash, a rounded body of doubtful nature. He kindly obtained for us other examples of *C. styx* from the locality in which he had collected the specimen referred to. These proved to contain the vegetative phases of the parasite described in this account.

† Most of the batches of *C. gallinæ* examined were from the nests of the blackbird and the thrush, and were found to be either free from Gregarines or infected in very small degree only. But a heavily infected colony was found in the nest of a blue-tit, taken

C. gallinulæ, Dale, from nests of the chaffinch and blackbird; *Ctenocephalus canis*, Curtis, from rabbits; and *Pulex irritans*, Linn., from a dog-kennel. All the series of *C. styx* dissected proved to be heavily infected, the percentage carrying Gregarines ranging from 65 to 100. The degree of infection was much less in the case of *C. farreni* and *C. gallinæ*—about 7 per cent. in the former and 5 per cent. in the latter—while the remaining species above mentioned were found to be free from Gregarines.

There are four previous records of the occurrence of Gregarines in fleas. R. Leuckart* mentioned that he had observed Gregarines in the gut of flea-larvæ, but gave no other information regarding them. E. H. Ross† found specimens, for which he proposed the name *Gregarina ctenocephali canis*, in the alimentary canal of adult examples of the dog-flea—"*Ctenocephalus serraticeps*," collected in Egypt. He described the trophozoites, which were frequently found in pairs, as rostrated and pear-shaped, and gave a brief account of the life-history of the parasite, at least, of those phases which occur in the adult flea. The description given is, however, not sufficient to permit the organism to be identified with certainty, but several of the features mentioned by Ross indicate that the "*Gregarina*" which he observed is distinct from the organism which forms the subject of the present communication. The third record is by Wellmer,‡ in the following terms: "*Actinocephalus parvus*, n. sp., im Darm der Larven von *Ceratophyllus fringillæ* (Wlk.) und *C. gallinæ* (Schrank). Das auf einem kurzen Halse des Protomerites sitzende, lange beständige, scheibenförmige Epimerit, trägt 8 Haken; Maximallänge der Sporonten 140 μ." This Gregarine is markedly different from ours in the nature of the armature of the epimerite and in other respects. The fourth record, by C. Strickland,§ relates to a Gregarine which lives for part of its life in the alimentary tract of the larva of *Ceratophyllus fasciatus*, and "for the other part lives freely in the excrement of this host." Strickland states that "the form of its epimerite and spores|| precludes us from placing it in any of the known families of

from a hole in a wall. This nest, in regard to protection and supply of moisture, was comparable to those of the sand-martin, and, like the latter, was evidently favourable to the survival of the spores of the Gregarine and to the chances of infection of the flea-larvæ.

* 'Arch. f. Naturgesch.,' 26 Jahrg., 1861, Bd. 2, p. 263.

† 'Ann. Trop. Med.,' Liverpool, 1909, vol. 2, pp. 359–363.

‡ 'Zool. Anz.,' 1910, Bd. 35, p. 533. Wellmer has since figured a cephalont and a sporont of *Actinocephalus parvus* in his Inaug. Diss., 'Sporozoen Ostpreussischer Arthropoden,' Königsberg, 1911, p. 33. This memoir is also published in 'Schr. Physik.-ökonom. Ges.,' Königsberg, Jahrg. 52, 1911, Heft 2.

§ 'Camb. Phil. Soc. Proc.,' 1912, vol. 16, pp. 460, 461.

|| No details of the forms of these are given.

cephaline Eugregarines, which have spores unarmed with spines." He proposes to name the Gregarine *Agrippina bona*, and to refer it to a new family—the Agrippinidæ. As this Gregarine exhibits features so striking as to require for its reception a new family, it is evidently different from that described below, for the latter is referable to a well-known genus, *Steinina*. *Agrippina bona* differs from the new species of *Steinina* described in the following pages, not only in certain points of structure, but in being restricted to the larval gut and fæces, for, in *S. rotundata*, the trophozoites, although occurring in the larva, reach their full size only in the adult flea.

Description of Steinina rotundata, *and of its Life-History.*

The life-cycle of this Gregarine has been traced almost exclusively in specimens of *Ceratophyllus styx*. The early trophic phases of the Gregarine occur in the larvæ of this flea, and are either attached to the wall or lie free in the lumen of the mid-gut. We have carefully examined the freshly dissected mid-gut, and also serial sections of the gut of many infected larvæ and adults, but we have not been able to identify an intra-cellular phase of the Gregarine. This stage, if it exists, will be extremely difficult to recognise with certainty, owing to the close resemblance between the young Gregarines and the smaller cells about the bases of the gastral epithelium, especially in the neighbourhood of the numerous crypts of regeneration. If there be an intra-cellular stage, it is of brief duration only, for we have observed a number of very young, ovoid trophozoites, 10 μ long and 5 μ broad,* attached by their narrower ends to the epithelial cells and hanging into the lumen of the mid-gut (Plate 1, fig. 1).

Average specimens of the Gregarine, from the mid-gut of young adult fleas, are 45 to 70 μ long and 30 to 50 μ broad, but, when full grown, the parasite attains a length of 180 μ and a breadth of 70 to 80 μ. It is usually differentiated into three regions—epimerite, protomerite, and deutomerite.

The epimerite varies considerably in shape. It often has the form of a blunt and flattened cone (figs. 2A, 3, 5, 6), on the apex of which a small pointed process may be present (fig. 3). In some specimens the epimerite is discoidal or saucer-shaped (fig. 4), its edge being slightly upturned (that is, away from the protomerite). The margin of the epimerite is rarely entire; sometimes it is lobate, but more usually it is fringed with short processes, rounded or pointed at their tips. These processes are, in some specimens, placed at almost regular intervals and form a single series, while in others they are less evenly arranged, and may be in two or three irregular rows (figs. 2, 4, 5, 6).

* The sporozoite is about 10 μ long and 1·5–2 μ broad (see p. 36).

The protomerite is narrowed in front into a neck-like region, which bears the epimerite, but the "neck" is usually short, and it is very short in the case of specimens lying free in the gut. The protomerite and deutomerite together form a mass which, in the smaller trophozoites, is usually oval, or, in some cases, nearly spherical, but in the largest examples is almost pear-shaped (fig. 8).

These two regions are separated internally by a thin septum, but the external surface does not bear any well-marked groove indicating the line of separation. In the few cases in which an external groove was present, it was faint. The septum is seldom equatorial in position; it is generally nearer the epimerite.* Below the thin epicyte (cuticle) there is a peripheral layer of ectoplasm 2 to 3 μ in breadth, which is clear, as it contains few granules. This layer is thicker at the distal end of the deutomerite. The protoplasm of the "neck" is generally homogeneous, like the ectoplasm. In this region faint longitudinal striæ, probably myocyte fibrillæ (myonemes), are present (fig. 9). Such striæ were not observed in other portions of the Gregarine. The endoplasm is very fluid, and contains numerous grey, refringent, nutrient granules, the largest of which are about 2 μ in diameter. Granules are much more abundant and coarser in the endoplasm of the deutomerite than in that of the protomerite. The nucleus is invariably situated in the deutomerite. In the fresh condition it is a clear vesicle with regular outlines, and contains from one to four karyosomes. The latter are usually homogeneous, but sometimes they are vacuolated (figs. 4, 5, 9), and in several cases they were observed to contain spherules of more refringent nature (fig. 6), composed of denser chromatin.

The Gregarines generally occur in considerable numbers; in one case 75 were present in the mid-gut, but they are not found in pairs, except at the moment of encystment.

When full grown the Gregarines become associated in pairs, preparatory to the formation of gametes. We have made numerous attempts to obtain the early stages of this association, but in the youngest met with the organisms had formed the common cyst wall, and the peripheral protoplasm of each individual was already raised into the rounded masses, which would soon have become gametes. We conclude that the association of the two

* We have seen a considerable number of trophozoites of various sizes up to 40 μ in length, in which the epimerite was wanting. These were lying free in the cavity of the mid-gut. They were ovoid in form (fig. 2, B), and the septum was almost invariably absent. Whether such specimens can subsequently develop a new epimerite, and re-attach themselves to the gut-wall, was not ascertained with certainty, but it appears probable that the smaller ones, at any rate, may do so.

individuals, and the formation of the cyst wall and of the gametes succeed each other very rapidly in this species.

A period of about two months elapses between the time of infection and the first appearance of cysts. This was ascertained by examination at regular intervals of the series of larval, pupal, and adult fleas, which were infected in the laboratory (see below, p. 36). Young larvæ, recently hatched, were collected on August 5, 1910, and placed, on August 8, in the *débris* of a nest known to be infected with the Gregarine. During the fortnight following August 10, 14 larvæ were examined; in all of them early trophic phases of the parasite were present. On August 25, cocoons were noticed; they contained either pupæ or scarcely formed adults. On August 26, 27, and 30, young adult fleas were removed from the cocoons and dissected, but only early vegetative phases of the Gregarine were found. Other adult fleas from this series, dissected at intervals during September, also exhibited trophic phases of the parasite. The first cysts were observed on October 15, that is, more than nine weeks after the larvæ had been placed in the infective environment.

The cysts are approximately spherical (fig. 10), and range from 110 to 185 μ in diameter.* When first formed they are translucent and grey, but they become finally yellow or yellowish-brown in colour. A mucous portion of the cyst envelope (*i.e.* the epicyst) appears to be absent, but the endocyst is well developed. When freshly formed, and for a considerable time afterwards, the endocyst is about 8 μ thick, but in very old cysts it has evidently undergone condensation, becoming thinner (1 to 2 μ), yellow, and less translucent. The cyst envelope is plain, that is, not sculptured in any way. The two individuals associated in each cyst give rise to gametes of apparently similar form, but we have not investigated the gametes, nor have we observed their fusion.

Each cyst soon contains numerous spores, among which are one or more masses of residual protoplasm, situated in the centre of the cyst. Each spore is oval, and is 11 to 12 μ long and about 7 μ broad (fig. 11). The epispore is closely applied to the endospore and exhibits two polar thickenings. Each spore, when ripe, contains eight sporozoites and a small amount of axial residual protoplasm.

Cysts—1 to 14 in number, in different individuals—occur lying freely in the mid-gut of the flea. They cannot escape entire from the digestive tract,

* We have occasionally seen small cysts, about 50 μ in diameter, but these did not contain spores. Probably each cyst resulted from the encystment of a single individual, and none of them would have developed further. The contents of two were examined, and were found to have degenerated.

as the lumen of the intestine is much too small to permit their passage (fig. 10). When fully ripe, the cysts burst in the mid-gut, and their contained spores are discharged with the fæces of the host. We have seen, in the mid-gut of an adult flea, a ruptured, yellow cyst, from which some of the spores (containing fully formed sporozoites) had escaped. The spores are dropped by the host, and are ingested by the flea-larvæ feeding among the *débris* in the nest. We have several times found young trophozoites in the mid-gut of larvæ which had been hatched only two or three days.

During the course of our experiments we procured a heavily infected series of adult fleas, by rearing them in the laboratory, from larvæ which we placed in an old nest, in which a considerable number of infected fleas had been kept and had died. Fourteen of these larvæ were dissected, and all proved to be infected; the rest were allowed to grow into adults, 11 of which were dissected, and were all found to contain Gregarines. As six adult fleas from the original nest in which the larvæ were found, proved on dissection to be uninfected, we think that we may reasonably attribute the occurrence of Gregarines in every example of our laboratory-bred series to the fact that the larvæ had been reared in material which offered them a ready means of infection.

Ripe spores, when placed in the fluid obtained by puncture from the mid-gut of larval fleas, soon exhibit changes. The polar caps of each spore become swollen and detached, leaving the rest of the spore like a barrel open at both ends, from which the eight sporozoites escape.

Each sporozoite is, when extended,* about 9·5 to 10·5 μ long, and 1·5 to 2 μ in diameter at its widest part (figs. 12, 13). It has a slender, finger-shaped, and mobile "rostrum," the organ by means of which fixation to the gastral epithelium of the host is accomplished. The protoplasm of the sporozoite is finely granular, and the nucleus is visible in the living condition. On fixing and staining the sporozoites, the protoplasm was found to stain almost homogeneously, except at the base of the "rostrum," where it was, in most cases, coloured rather more deeply. The nucleus is large and vesicular. Associated with its thick membrane are a few (four to six) small and deeply stained chromatin masses. A karyosome is not present at this stage.

We have not been able to observe the attachment of the sporozoite to the epithelium of the larval gut, nor (as stated on p. 33) to determine whether or not this is followed by an intracellular stage. There is evidence, however, that the sporozoite, on fixation to the host, becomes more ovoid, that is, shorter but broader, for, on the wall of the mid-gut of a young larva (1·5 mm. long), we found a very young trophozoite, about 5 μ long and 3 μ broad. Whether this was attached to, or was within, an epithelial cell

* The body of the sporozoite can be bent into a curve or hook (fig. 12).

could not be ascertained. The karyosome, which was already developed in this young trophozoite, soon attains a considerable size. The septum, dividing the protomerite from the deutomerite, appears later, when the organism has reached a length of about 30–40 μ.

During the early phases of growth in the larval gut, the trophozoite may relax its hold of the epithelium, and may, later, renew its attachment, but whether all the detached trophozoites succeed in refixing themselves is not clear (see footnote, p. 34).

Systematic Position.

The characters of the vegetative phases, cysts, spores, and sporozoites of the Gregarine described in the preceding pages agree closely with those of the genus *Steinina*, Léger et Duboscq,* which was defined in the following terms: "Polycystidée de la famille des Actinocéphalides, caracterisée par un épimérite constitué d'abord par un court prolongement digitiforme et mobile et plus tard par un bouton aplati. Développement à phases fixées pouvant alterner avec des phases libres. Kystes sans sporoductes, à sporocystes biconiques, fortement ventrus." The only feature in which the organism described by us deviates from this diagnosis is the shape of the spore, which is not strongly inflated. However, the difference in regard to this character is so small that we do not hesitate to refer our Gregarine to the genus *Steinina*. This genus has hitherto been represented by a single species —*S. ovalis* (Stein)—recorded† from the intestine of the larva (meal-worm) of *Tenebrio molitor*. Our specimens differ from this species in (1) the shape of the spores, which are less strongly inflated; (2) the size of the spores, which are 11 to 12 μ long and about 7 μ broad (in *S. ovalis* they are 9 μ long and 7·5 μ broad); and (3) the younger trophic phases are usually less elongate and more oval or spherical, and the sporonts ("sporadins") are more pyriform. These differences seem to us to be sufficient to justify the formation of a new species, for which we propose the name *Steinina rotundata*, for the specimens described in this paper.

Steinina rotundata, n. sp.—Near *S. ovalis*, Léger et Duboscq, but the spores are larger and less strongly inflated than those of the latter. In the younger trophozoites, the protomerite and deutomerite together usually form an oval or sub-spherical mass, but later the organism becomes more or less

* 'Archiv f. Protistenkunde,' 1904, vol. 4, pp. 352—354, 2 figs.

† See Léger and Duboscq, *op. cit.*, in which the earlier records are cited. *S. ovalis* has also been recorded from the gut of the meal-worm by Kuschakewitsch ('Archiv f. Protistenkunde,' 1907, Suppl. vol. 1, p. 203) and Pfeffer (*op. cit.*, 1910, vol. 19, p. 108), and its cycle of development has been studied by Mavrodiadi ('Procès-verbaux Soc. Natural. Varsovie,' 1909, vol. 21, pp. 106—118).

pyriform. Adults solitary, except when association in pairs occurs preparatory to the formation of gametes. Cysts spherical, 110 to 185 μ in diameter, without sporoducts. Spores oval, 11 to 12 μ long, and about 7 μ broad.

Habitat.—The mid-gut of certain species of bird-fleas of the genus *Ceratophyllus.*

DESCRIPTION OF PLATE.

List of Reference Letters.

A., anus; Ep., epithelium of mid-gut; G., gizzard; Int., intestine; K., karyosome; M., muscle-fibre; M.G., mid-gut; M.T., Malpighian tube; N., nucleus; Œ., œsophagus; R., rectum; R.P., rectal papilla.

All the figures relate to *Steinina rotundata,* nov. sp., from the mid-gut of *Ceratophyllus styx,* Rothsch. Figs. 1, 9, and 13 are from stained preparations; the rest were drawn from living specimens.

Fig. 1.—Section of a portion of the epithelium of the mid-gut of a young larva, 3 mm. long, to which a young trophozoite is attached.

Fig. 2.—Two later trophic phases, from the mid-gut of a young adult flea. A exhibits the division into epimerite, protomerite, and deutomerite; B was lying free in the cavity of the gut and was not differentiated into regions.

Figs. 3, 4, 5, 6, 7.—Trophozoites from an adult flea. On dissection of the mid-gut these specimens became free from the gut-wall. Note the varying form of the epimerite, the vacuolation of the karyosome in figs. 4, 5, and the denser chromatin-spherules in the karyosome of fig. 6.

Fig. 8.—Almost full-grown adult (sporont or sporadin), found attached to the gut-epithelium of an adult flea. Note the magnification is only half that of figs. 3 to 7 and 9.

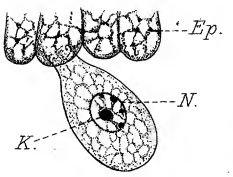
Fig. 9.—Section of a portion of the wall of the mid-gut of an adult flea, in the epithelium of which the epimerite of a well-grown trophozoite is deeply embedded.

Fig. 10.—Outline of the gut—from the œsophagus to the anus—of an adult flea and of its contained parasites. Only the proximal portions of the Malpighian tubes are shown. The five cysts contained fully formed spores. The four trophozoites were similar in form to that shown in fig. 2, B.

Fig. 11.—Outline of the wall of a spore.

Fig. 12.—A sporozoite, immediately after its liberation from the spore, drawn while living. Note the slender, mobile "rostrum." The opposite end of the sporozoite was bent into the form of a hook.

Fig. 13.—Sporozoites, from a preparation fixed with osmic vapour and stained with Leishman's stain. A, B, C, D, represent typical sporozoites; those lettered E, F, G, H are less usual forms.



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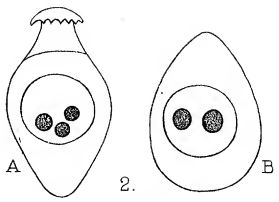

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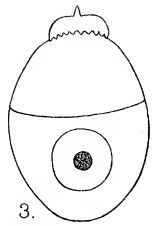

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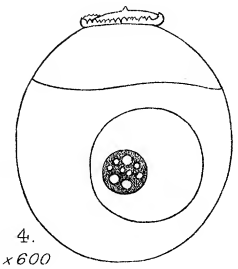

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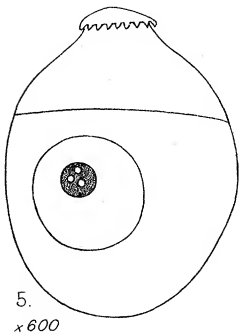

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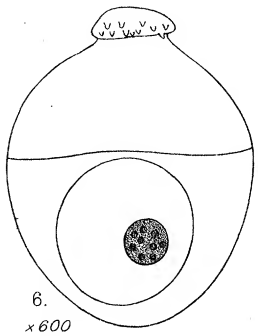

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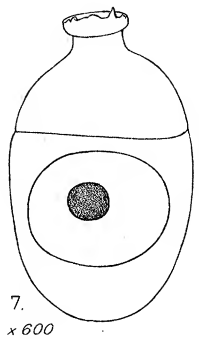

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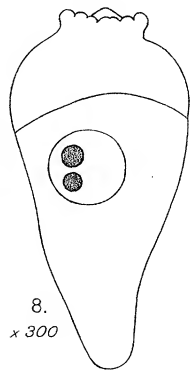

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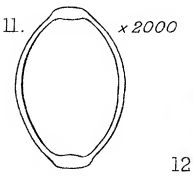

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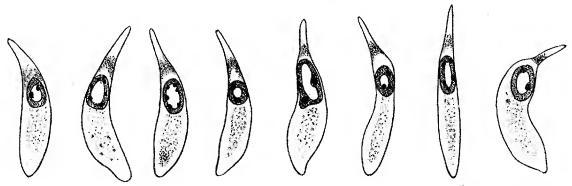

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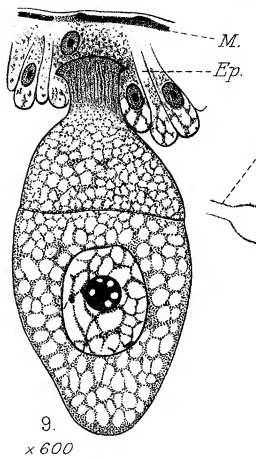

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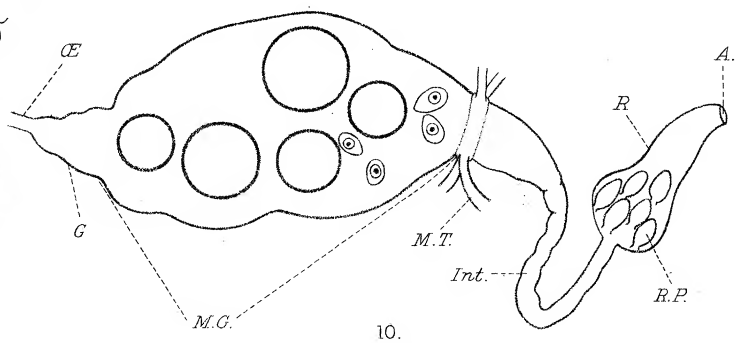

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